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Postharvest ethanol and hot water treatments of table grapes to control gray mold

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Abstract

Complete inhibition of the germination of spores of *Botrytis cinerea* occurred after a 10 s exposure to 30% ethanol or more at 24 °C. Mortality of spores in heated 10% ethanol was higher than in water at the same temperatures. Immersion of naturally infected, freshly harvested table grapes for 30 s in 30% ethanol at 24 °C reduced decay approximately 50% after 35 days of storage at 1 °C. The addition of ethanol significantly improved the efficacy of a hot water treatment applied to grapes that were inoculated with *B. cinerea* two hours prior to immersion in heated solutions. Immersion of inoculated, freshly harvested table grapes for 3 min at 30, 40, or 50 °C in 10% ethanol reduced decay to 20.7, 6.7, and 0.1 berries/kg after 30 days of storage at 1 °C, while decay after immersion in water at these temperatures was 35.9, 17.6, and 1.7 berries/kg, respectively. Immersion for 30 or 60 s at 50, 55, or 60 °C in water or 10% ethanol also significantly reduced the number of decayed berries that developed after storage for 30 days at 1 °C. The appearance of the rachis and berries, incidence of cracked berries, flesh browning, flavor, weight loss, and berry color were examined and most treatments did not adversely affect these quality parameters. Prompt drying of the fruit after treatment was important to prevent berry cracking.

Keywords: *Vitis vinifera*; *Botrytis cinerea*; Dip; Heat treatments; Alcohol

1. Introduction

Gray mold, caused by *Botrytis cinerea* Pers.:Fr., is the most economically important postharvest disease of table grapes (Cappellini et al., 1986). *B. cinerea* is troublesome because of its ability to develop at temperatures as low as −0.5 °C and proliferate by mycelial growth from berry to berry. The disease cannot be sufficiently controlled by cooling alone (Bulit and Dubos, 1998).

The standard practice to control postharvest decay of grapes worldwide is to fumigate the fruit after harvest with sulfur dioxide gas, either by repeated application of gas in storage rooms, or to fumigate packed fruit in polyethylene-lined boxes with continuous-release sulfur dioxide generator

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pads. Problems associated with sulfur dioxide use are bleaching and other injuries to the rachis and berries, excessive sulfite residues that can accumulate in grapes after prolonged and frequent fumigation, and issues of gas storage, corrosion of equipment within storage facilities, worker safety, and air quality (Smilanick et al., 1990; Crisosto and Mitchell, 2002).

Ethanol is a common food component with potent antimicrobial activity (Larson and Morton, 1991). Ethanol dips and vapors were reported to control postharvest diseases of peaches, citrus fruit, and table grapes (Larson and Morton, 1991; Feliciano et al., 1992; Smilanick et al., 1995; Mlikota Gabler and Smilanick, 2001; Mlikota Gabler et al., 2002), especially when heated (Smilanick et al., 1995; Margosan et al., 1997). The beneficial effect of immersion in hot water to control postharvest diseases has been shown with various fruit varieties, but not table grapes (Madhukar and Reddy, 1990; Barkai-Golan and Philips, 1991; Cheah et al., 1992; Jacobi and Wong, 1992; Garcia et al., 1995; Schirra et al., 1996; Schirra and Ben-Yehoshua, 1999).

Our objectives were to evaluate the efficacy of brief immersion in ethanol or hot water, alone or in combination, to control gray mold on table grapes, and to determine the impact of these treatments on fruit quality.

2. Materials and methods

2.1. Fruit

'Crimson Seedless', 'Flame Seedless' and 'Thompson Seedless' grapes were harvested from vineyards located in the San Joaquin Valley in central California (USA). 'Muskule' grapes were harvested from a vineyard located in Iznik region in Bursa (Turkey). The grapes were used on the day of harvest.

2.2. Fungi

Botrytis cinerea was isolated from infected grape berries and cultured on potato dextrose agar (PDA, Difco, Detroit, USA). Spores were harvested from 2-week-old PDA cultures of *B. cinerea* grown at 25 °C. Five milliliters of sterile water, containing 0.005% (v/v) Triton X-100, was added to a Petri plate culture, the spores were gently dislodged from the surface with a sterile glass rod, and suspensions were filtered through three layers of cheesecloth to remove mycelial fragments. The suspensions were diluted with sterile water to an absorbance of 0.25 at 425 nm as determined by a spectrophotometer. This density contained 1.2×10^6 conidia/ml. Further dilutions with sterile water were made to obtain the desired spore concentrations.

2.3. Ethanol toxicity to B. cinerea

Two methods were used to determine the effect of ethanol on the germination of spores of *B. cinerea*. In the first method, spores (12,500 spores/ml) were mixed with various ethanol concentrations at room temperature (22–24 °C) in a final volume of 2 ml. After 10 s, the spore suspensions were diluted 100-fold in sterile water and 10 μl was plated on PDA. After 48 h incubation at 24 °C, the colonies per plate were counted. Data were expressed as the percentage of germinated spores. In the second method, survival of spores in heated water or 10% ethanol solution was determined. Sterile screw-capped glass tubes containing 9 ml of sterile water or 100% ethanol were placed in a water bath at 30, 50, 55, or 60 °C, and allowed to equilibrate. Then, 1 ml of concentrated *B. cinerea* spore suspension was added to each tube, to achieve a final concentration of 28,000 spores per ml. After 30 or 60 s, tubes were removed from the water bath and immediately placed on ice. Aliquots of 100 μl of each suspension were plated onto PDA plates. After 72 h incubation at 24 °C, the colonies per plate were counted. Data were expressed as the percentage of germinated spores.

2.4. Ethanol treatment of table grapes

Entire clusters of grapes were harvested and placed in ventilated polyethylene (VPE) bags containing about 500–700 g of grapes each. The grapes were not inoculated. In the first test, 'Crimson Seedless' grapes were immersed in water alone or in 30 or 60% ethanol solutions for 30 s at 24 °C. In the second test, 'Flame Seedless' grapes were immersed in water alone or 20, 30, 40, or 50% ethanol solutions for 30 s at 24 °C. The fruit were immersed in the solutions while contained within the VPE bags. Four replicates were treated with 'Crimson Seedless' grapes, each

replicate consisting of 4.2 kg of grapes divided among six VPE bags. Three replicates were treated with 'Flame Seedless' grapes, each replicate consisting of 2.4–2.8 kg of grapes divided among four VPE bags. After treatment, the fruit were removed from the bags, dried in open air under shade for about 30 min, and placed into new VPE bags, placed in fiberboard boxes, stored at 0–1 °C (RH > 90%) for 24 h to facilitate rapid cooling, then wrapped with polyethylene stretch film (20 μm) to minimize weight loss and stem desiccation and stored for four weeks at 0–1 °C (RH > 90%). The number of decayed berries per kg of fruit was recorded after storage.

Table grapes inoculated with *B. cinerea* were immersed in ethanol using the materials and methods of previous experiment with three modifications: (1) whole clusters of table grapes were inoculated by spraying 300 ml of a spore suspension (12,500 conidia/ml) of *B. cinerea* to 120 kg fruit with a compressed air sprayer, and then left to air dry at 1 °C for 12 h before treatment; (2) inoculated bunches were warmed at 24 °C for 1 h before the treatments; and (3) the ethanol solution was heated for most treatments.

Three experiments were conducted. In the first experiment with 'Thompson Seedless' grapes, clusters were dipped into 10% ethanol or water for 3 min at 40 or 50 °C. In the second and third experiments with 'Crimson Seedless' and 'Muskule' grapes, the duration of immersion was 30 or 60 s and the temperature was 50, 55, or 60 °C.

2.5. Grape quality

The quality of the grapes was assessed on inoculated bunches at the end of storage by evaluation of berry and rachis appearance, incidence of cracked, brown, and shattered berries, berry color, taste, and weight loss. The evaluation methods and the units employed are described in Table 1.

2.6. Statistical analysis

An analysis of variance was applied to the results of each experiment. Incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before analysis. Means were separated using Fisher's LSD ($P < 0.05$).

Table 1
Quality parameters and methods employed to evaluate table grape quality after storage for one month at 1 °C

Quality parameters	Methods of evaluation and units
Weight loss	Percentage of difference between the weight of 9 clusters (each 500–800 g) before and at the end of storage.
Rachis appearance	Visual index of 9 clusters (each 500–800 g): fresh and green = 1, green = 2, semidry = 3, 50% dry = 4, completely dry = 5.
Berry appearance	Visual index of 9 clusters (500–800 g): excellent = 1, good = 2, slightly dull = 3, < 50% brownish and soft berries = 4, > 50% brownish and soft berries = 5.
Shatter	Number of shattered berries/kg.
Cracking	Number of cracked berries/kg.
Browning	Number of brown berries/kg.
Taste	Evaluated by a panel according to hedonic scale (1–10).
Berry color	Color recorded as CIELab color space determined with a Minolta CR-200 surface colormeter (Minolta Corp. Ramsey, N.J.) of 5 randomly selected berries per cluster. Decayed berries were omitted.

3. Results

3.1. Spore mortality

An exposure of 10 s of *B. cinerea* spores at 22–24 °C to ethanol at 30% or more completely inhibited spore germination (Fig. 1). Spores similarly immersed in 10 or 20% ethanol germinated at 93 and 78%, respectively. Heating the 10% ethanol solution greatly increased the inhibition of spore germination (Fig. 2). At 30 °C, more than 80% of the spores germinated after a 30 s immersion in water or ethanol at 10%, while at 50 °C, only those immersed in water germinated and those in 10% ethanol were completely inhibited. At 55 °C for 30 s, 18% of the water-treated spores germinated, while none of the ethanol-treated spores did. No germination occurred after immersion in water or 10% ethanol at 60 °C for 30 s. At 30 °C, more than 80% of the spores germinated after a 60 s immersion in water or ethanol at 10%. After 60 s immersion in water at 50 °C, 15% of the spores germinated, while none germinated after immersion in water at 55 or 60 °C. After 60 s immersion in 10% ethanol at 50, 55, or 60 °C, none of the spores germinated (data not shown).

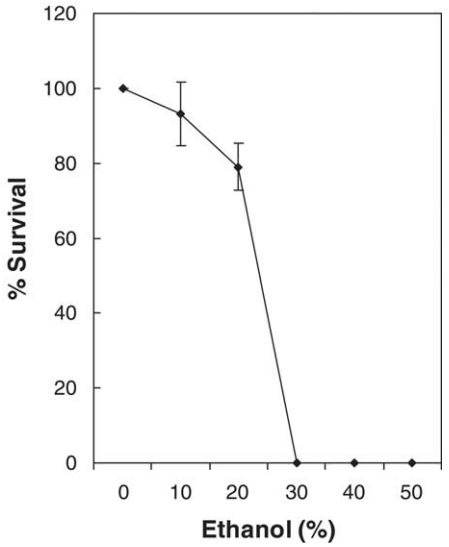

Fig. 1. Germination on potato dextrose agar of spores of *B. cinerea* after immersion in ethanol for 10 s at 22–24 °C.

3.2. Decay control

The natural incidence of decay, most all of which was gray mold, was significantly reduced by ethanol treatment of 'Crimson Seedless' and 'Flame Seedless' grapes. Immersion of 'Crimson Seedless' grapes in 30 or 60% ethanol reduced the decay of berries similarly and by about 50% (Fig. 3). Immersion of 'Flame Seedless' grapes in 30, 40, or 50% ethanol reduced the decay of berries similarly and by about 50%, while immersion in 20% ethanol was not significantly different from the control (Fig. 3).

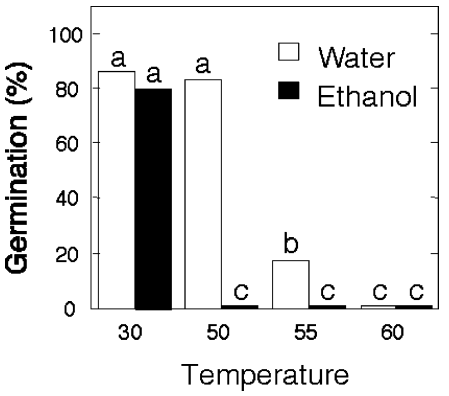

Fig. 2. Germination on potato dextrose agar of spores of *B. cinerea* after immersion in water or 10% ethanol for 30 s at 30, 50, 55, or 60 °C. Columns with unlike letters differ significantly ($P < 0.05$).

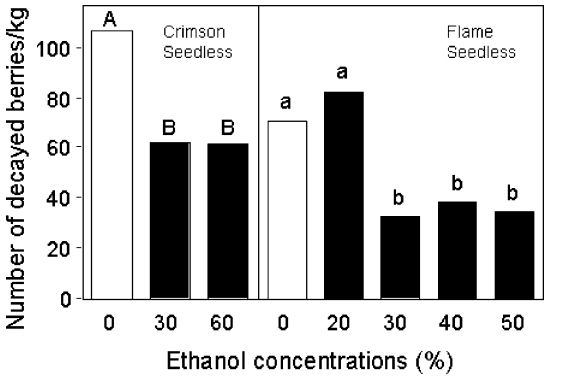

Fig. 3. Natural incidence of gray mold, caused by *B. cinerea*, on 'Crimson Seedless' and 'Flame Seedless' grapes after immersion in water or ethanol solutions for 30 s and storage for 1 month at 1 °C. Columns within each cultivar with unlike letters differ significantly ($P < 0.05$).

Control of decay of 'Thompson Seedless' grapes inoculated with spores of *B. cinerea* before immersion for 3 min in water alone or with 10 or 35% ethanol, was improved significantly by the addition of ethanol to the water or increasing the solution temperature (Fig. 4). Then immersion in water alone at 30, 40, and 50 °C reduced the number of decayed berries to 35.9, 17.6, and 1.7, respectively, while 10% ethanol at these temperatures reduced the number of decayed berries

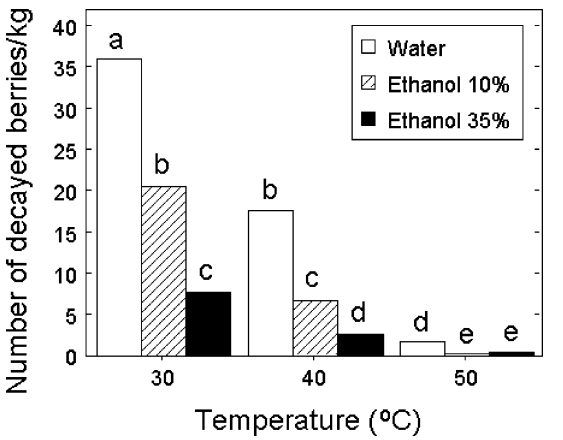

Fig. 4. Incidence of gray mold on 'Thompson Seedless' grapes inoculated with spores of *B. cinerea* prior to immersion for 3 min in heated water or ethanol and storage for 1 month at 1 °C. Columns with unlike letters differ significantly ($P < 0.05$).

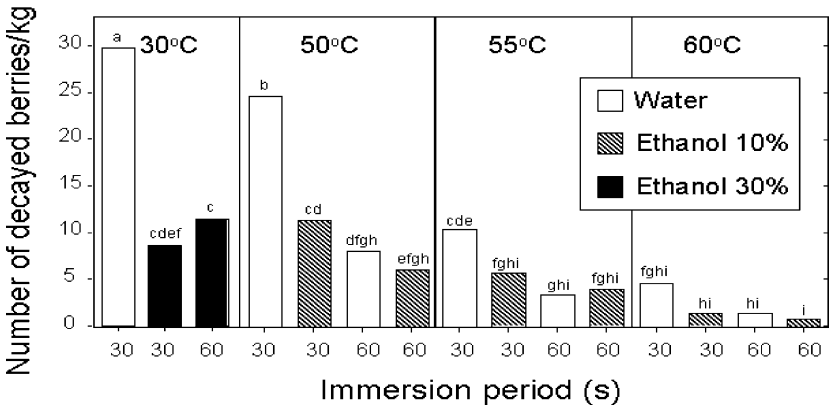

Fig. 5. Incidence of gray mold on 'Crimson Seedless' grapes inoculated with spores of *B. cinerea* prior to immersion for 30 or 60 s in heated water or ethanol and storage for 1 month at 1 °C. Columns with unlike letters differ significantly ($P < 0.05$).

per kg to 20.7, 6.7, and 0.1, respectively. Immersion in 35% ethanol solution at 30 °C reduced the number of infected berries to 7.7 per kg, and was as effective as 10% ethanol at 40 °C. At 50 °C, ethanol treatments nearly eliminated decay, while two infected berries per kg developed after treatment with water alone at this temperature (Fig. 4). Control of decay of 'Crimson Seedless' grapes, inoculated with spores of *B. cinerea* before immersion for 30 or 60 s in water alone or with 10% ethanol at 50, 55, or 60 °C, or at 30 °C in water or 30% ethanol, was improved significantly by the addition of ethanol to the water, increasing the solution temperature or prolonging the immersion period (Fig. 5). Immersion for 30 s in 10% ethanol at 50 or 55 °C was significantly better than water alone at these temperatures. Immersion of grapes in 30% ethanol at 30 °C was significantly less effective than immersion in 10% ethanol at 60 °C. The highest efficacy on 'Muskule' grapes inoculated with *B. cinerea* was achieved by 10% ethanol treatments at 55 and 60 °C for 30 s and water treatment at 60 °C for 30 s (Fig. 6).

3.3. Quality evaluation

The quality of 'Thomson Seedless' and 'Crimson Seedless' grapes after treatment with heated water or ethanol and followed by one month storage at 1 °C are shown in Tables 2 and 3, respectively. The loss in weight of 'Thompson Seedless' grapes during storage after treatment with heated water or ethanol for 3 min at 40 or 50 °C was not significantly greater than that of the control fruit with the exception of the treatment with 35% ethanol at 50 °C (Table 2). Rachis and berry appearance, cracked berries, taste, and color were not affected by these treatments. Shatter was significantly higher among control grapes and often associated with decayed berries. Browning, also often associated with decayed berries, was significantly higher among control grapes than among many of the treated berries. The loss in weight of 'Crimson Seedless' grapes during storage after treatment with heated water or ethanol for 30 or 60 s at 50, 55, or 60 °C was occasionally significantly greater than that of the control fruit, although the magnitude of the differences were small

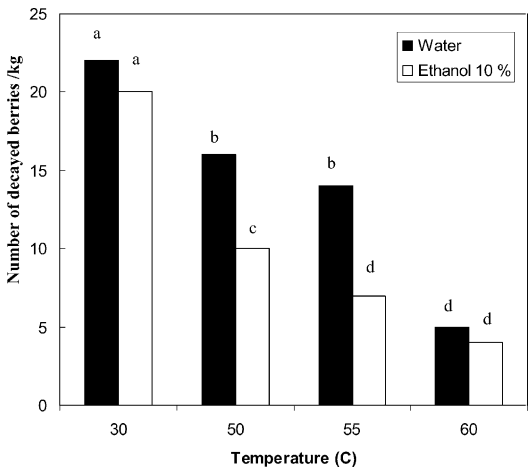

Fig. 6. Incidence of gray mold on 'Muskule' grapes inoculated with spores of *B. cinerea* prior to immersion for 30 s in heated water or ethanol and storage for 1 month at 1 °C. Columns with unlike letters differ significantly ($P < 0.05$).

Table 2
Quality of 'Thompson Seedless' table grapes after immersion in the given solutions for 3 min followed by 1 month of storage at 1 °C

Ethanol (%)	Temperature (°C)	Weigth Loss (%)[a]	Rachis appearance	Berry appearance	Shatter	Cracking	Browning	Taste	Berry color		
									L	a	b
0	30	5.2 b	2.6 ab	2.6 ab	40.3 a	6.3 a	12.1 a	7.6 b	39.3 a	7.1 a	15.7 a
10	30	6.1 b	2.6 ab	2.0 b	16.3 b	7.9 a	8.8 ab	8.3 ab	40.8 a	5.7 a	13.6 a
35	30	6.3 b	2.6 ab	2.0 b	21.6 b	1.5 a	4.1 bc	8.8 a	40.4 a	5.9 a	13.7 a
0	40	5.2 b	2.0 b	4.0 a	7.9 b	4.6 a	9.3 ab	8.0 ab	40.7 a	6.5 a	14.7 a
10	40	5.1 b	2.6 ab	3.0 ab	12.3 b	6.4 a	6.3 abc	8.0 ab	40.9 a	6.6 a	14.5 a
35	40	5.9 b	4.0 a	3.0 ab	16.7 b	7.5 a	3.8 bc	8.3 ab	38.4 a	6.4 a	14.3 a
0	50	5.6 b	3.3 ab	3.3 ab	18.6 b	0.5 a	3.0 bc	8.5 ab	39.4 a	5.8 a	12.2 a
10	50	5.3 b	2.6 ab	2.3 b	5.0 b	5.7 a	1.6 bc	8.5 ab	44.4 a	7.3 a	17.1 a
35	50	8.0 a	4.0 a	3.3 ab	14.6 b	2.4 a	0.9 c	8.5 ab	38.3 a	5.6 a	14.1 a

The methods of evaluation and units of each measurement are described in Table 1. Values in columns not followed by letters were not significantly different.

[a] Values in columns followed by unlike letters differ significantly according to Fisher's LSD ($P < 0.05$).

Table 3
Quality of 'Crimson Seedless' table grapes after immersion in the given solutions followed by 1 month storage

Ethanol (%)	Temperature (°C)	Time (s)	Weight loss (%)[a]	Rachis appearance	Berry appearance	Shatter
0	30	30	2.3 e	3.0 de	2.1 de	30.5 a
0	50	30	2.8 bcde	3.6 abcd	3.1 ab	30.5 a
0	50	60	2.7 cde	3.2 bcde	2.5 bcd	12.1 cd
0	55	30	3.1 abcd	3.9 a	3.0 abc	22.4 b
0	55	60	3.3 abc	3.6 abcd	3.0 abc	7.9 d
0	60	30	3.0 bcd	3.9 a	2.4 cd	10.7 cd
0	60	60	2.5 de	3.9 ab	3.4 a	14.0 cd
10	50	30	2.9 bcde	3.2 cde	2.9 abc	15.6 bcd
10	50	60	2.7 cde	2.9 e	2.5 bcd	13.6 cd
10	55	30	3.7 a	3.7 abc	2.6 bcd	8.0 d
10	55	60	2.8 bcde	3.6 abcd	2.9 abc	11.1 cd
10	60	30	3.4 ab	4.0 a	3.0 abc	11.5 cd
10	60	60	2.5 de	3.8 abc	2.5 bcd	8.1 d
30	30	30	2.9 bcde	3.3 bcde	2.1 de	13.6 cd
30	30	60	2.7 cde	2.8 e	1.8 e	18.2 bc

The methods of evaluation and units of each measurement are described in Table 1.

[a] Values in columns followed by unlike letters differ significantly according to Fisher's LSD ($P < 0.05$).

and variable (Table 3). Rachis appearance was significantly harmed by 60 °C treatment with water or ethanol, and by some 55 °C treatments. Berry appearance was often significantly affected, particularly by treatments with water or ethanol at 55 or 60 °C, while the appearance of berries after immersion in unheated (30 °C) ethanol at the higher concentration (30%) for 30 or 60 s was significantly superior to all of the heated ethanol or hot water treatments. Shatter was significantly higher among control grapes and those treated for 30 s with 50 °C water, and often associated with a high incidence of decayed berries.

4. Discussion

Immersion of grapes in ethanol at ambient temperatures, or in heated ethanol at lower concentrations or heated water, significantly controlled postharvest gray mold of table grapes. Our work corroborates several studies that showed ethanol can control postharvest diseases of peaches, citrus fruit, and table grapes (Feliciano et al., 1992; Smilanick et al., 1995; Mlikota Gabler and Smilanick, 2001; Lichter et al., 2002; Mlikota Gabler et al., 2002), especially when heated (Margosan et al., 1994, 1997; Smilanick

et al., 1995). This is the first report where immersion of table grapes in heated ethanol or water was evaluated.

The germination of spores of *B. cinerea* immersed briefly in 10 and 20% ethanol at ambient temperatures was reduced, while immersion in 30% ethanol completely inhibited spore germination. In contrast to our findings, Lichter et al. (2002) reported that the concentration of ethanol that completely inhibited spore germination was 40%. Heating the ethanol solution greatly increased its toxicity to spores of *B. cinerea*. Spore germination was completely inhibited by a 30 s exposure to 10% ethanol at 50 °C, whereas exposures of similar duration in 10% ethanol at ambient temperature, or in water alone at 50 °C, were not inhibitory. Margosan et al. (1997) reported spores of *Monilinia fructicola* and *Rhizopus stolonifer* died about 10 times faster in heated 10% ethanol at 46 or 50 °C than in water at the same temperatures. The combined application of ethanol and heat to ungerminated spores of *B. cinerea* caused retardation of subsequent mycelial development (Lichter et al., 2003). Barkai-Golan and Philips (1991) stated that the increase in spore mortality that occurred when hot water and low ethanol concentration treatments were combined may be the result of their affecting the same sites in the spores. Since low concentrations of ethanol can lower the temperature at which phospholipids undergo a phase change (Rowe, 1983), the increases in the spore mortality and decay control following the addition of ethanol may have resulted from a lowering of the phase-change temperature of mitochondrial membranes of the spores under these conditions (Margosan et al., 1997).

At ambient temperatures, immersion in 30% or higher concentrations of ethanol modestly but significantly reduced the postharvest decay of 'Crimson Seedless' or 'Flame Seedless' table grapes, while 20% ethanol was ineffective. Ethanol concentrations of 30, 40, and 50% were equally effective, which supports our findings that spores of *B. cinerea* were completely inhibited by 30% and higher concentrations of ethanol. Similarly, Lichter et al. (2002) also reported that ethanol concentrations of 30 and 50% did not significantly differ in reducing gray mold on grapes.

At elevated temperatures, much lower concentrations of ethanol controlled gray mold effectively. Good control with water alone was also observed, but at higher temperatures than when ethanol was present. Immersion of 'Thompson Seedless' and 'Muskule' grapes, which are green in color, in heated 10% ethanol for 3 min at 50 °C completely controlled gray mold; immersion in water alone at this temperature was less effective but it markedly reduced gray mold. Neither of these treatments harmed the berries. Immersion of 'Crimson Seedless' grapes, which are red in color, in heated 10% ethanol for 1 min at 50 °C or higher effectively controlled gray mold; immersion in water alone at this temperature at 50 °C had little effect on gray mold incidence but it was effective at 55 °C or higher. Most quality aspects were unchanged, but berry and rachis appearance were affected, particularly after treatment at 55 or 60 °C in either water or ethanol. We attribute this to elevated heat, since no impact on berry appearance was observed when grapes were immersed in 30% ethanol at ambient temperature. Many fruit tolerate exposure to ethanol or its vapors without visible harm, although it can inhibit ripening of climacteric fruit (Paz et al., 1981; Margosan et al., 1997). Lichter et al. (2002) evaluated the quality of grapes when immersed in 35 or 50% ethanol at room temperature. They did not observe any negative effects on grape appearance or rachis desiccation during up to five weeks of storage; furthermore, in that study the flavor of ethanol-treated grapes was rated significantly better than that of the untreated control.

There are several practical issues to address before hot water or ethanol treatments could be commercially implemented. Ethanol is an inexpensive and well-studied natural substance present in many food products. The use of ethanol should be less than that of synthetic fungicides. It should pose a minimal ingestion hazard to humans because of its low mammalian toxicity. The flash point of ethanol, defined as the concentration at which ignition by flame is possible, is 24 °C for a solution containing 50% (v/v) ethanol. In California, table grapes typically are harvested and packaged within the vineyard of harvest, and they are stored and fumigated weekly with sulfur dioxide gas within these packages. This practice limits opportunities to apply wet treatments, unlike other fruit that are subjected to packinghouse washing prior to packaging. The natural appearance of berry surface waxes, termed 'bloom', becomes altered by physical contact and is perceived as less attractive,

so handling is deliberately minimized. Heated water and heated ethanol treatments altered the bloom and made the surface of the berries appear smoother and shinier. Unheated ethanol treatments themselves did not alter the bloom objectionably, although the handling to apply the treatments could. We and others (Phillips, personal communication) have observed that wet treatments of grapes, unless the berries are dried promptly, can cause some berries to crack. Excessive moisture within packaging (Ladania and Dhillon, 1989) and even rainfall near harvest can cause mature berries to crack (Uys and Calitz, 1997). In this work, we dried the berries carefully after all treatments, and if heated water or ethanol treatments were implemented commercially, controlled drying will be needed. Grapes are often stored for periods that exceed three months and adequate protection from gray mold is accomplished by repeated sulfur dioxide fumigation. Ethanol or hot water, applied only once soon after harvest, was effective during the one-month storage period in our tests, and for two months in the work of Lichter et al. (2002). However, if the stored grapes had numerous latent infections that emerged during storage, ethanol or hot water treatments could not control them while the repeated sulfur dioxide fumigations would suppress the development of these infections. Nevertheless, ethanol and hot water treatments could be effective for grapes not stored for prolonged periods.

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